

A CLINICAL STUDY OF BLOOD IRON AND HEMOGLOBIN.

BY C. W. DOWDEN, M.D., AND CLYDE MCNEILL, M.D.,

LOUISVILLE, KY.

With the Technical Assistance of J. D. MCNEILL, B.S.

We are approaching this subject with a healthy skepticism, born of the belief that, in spite of the great progress that has been made in hematology during the past few years, we are still surrounded by a mass of doubtful and uncertain data that leaves us far from a simple and satisfactory working basis. One must respect and accept as authoritative the original work of Haden,¹ Osgood and Haskins² and others for their valuable contributions. Let it be known also that since this is a clinical investigation we shall not consider much of their work, which has dealt largely with volume studies. We cannot but feel that our immediate clinical need is the adoption of simpler methods to remove us from much of the chaos that for the past few years has served to confuse us. We believe that for many years at least, the estimation of the number of red blood cells and the hemoglobin per given quantity of blood, as well as their relationship to each other will constitute the chief desideratum in hematologic studies. One must also give greater thought to the question of iron in its relationship to hemoglobin.

It seems unnecessary before this body of medical men to further stress the great importance of reporting the amount of hemoglobin in grams per 100 c.c. of blood, but a citation of a few of the existing errors will not be amiss. For instance one of the Sahli instruments is so graduated to indicate 100% hemoglobin as containing 17.2 grams, one of the Dare instruments to contain 13.8 grams of hemoglobin, representing a difference of more than 20%, perhaps better shown by the equation, viz.:

$$17.2 \text{ grams Sahli} = 100\%$$

$$17.2 \text{ grams Dare} = 124\%$$

It should be known also that there is a wide difference in various Sahli instruments as well as in the Dare instruments. Certainly percentage reports are meaningless, and even when accompanied by

the name of the method used are not accurate unless such instrument has been standardized with one of the more accurate methods to be mentioned. What should be accepted as a normal average figure for the number of grams of hemoglobin contained in 100 c.c. of blood, and what should be accepted as a normal average figure for the number of red blood cells per c.mm. of blood? Personally we believe that no single figure will serve the purpose with even a fair degree of accuracy. Such figures are ordinarily obtained by taking an average for a large number of so-called normals. In this number we will find a range of from 11.0 to 16.5 (a difference of 34%) grams of hemoglobin per 100 c.c. of blood, and a range of from 4,000,000 to 6,000,000 (a difference of 34%) for the number of red blood cells per c.mm. of blood. Certainly such variations are too great to permit of accurate averaging. The question naturally arises what is normal blood? An individual may be subjectively healthy and with perfectly normal functional tests of all kinds and still have comparatively low hemoglobin and red cell counts. Such findings are not uncommon among nurses, technicians, secretaries, etc., and such individuals constitute a large per cent of our so-called normal series. Conversely an individual may have advanced heart, kidney, lung, gastrointestinal or other pathological conditions with blood examinations much nearer the generally accepted average than the class previously mentioned (Chart No. 8). These individuals are not used in the normal series.

Both the quantity of hemoglobin and the number of red blood cells are variables, depending probably upon age, sex, climate, food, exercise and barometric pressure. One of us (J. D. Mc.N) examined the blood of one patient daily for a month and hourly for a day and found the red blood count to vary from 5,128,000 to 6,176,000 and the hemoglobin from 11.3 gms. to 15.3 gms. With such variations why not abolish such terms as "normal averages" and substitute "a normal range" for hemoglobin and red blood cells, as we do for the chlorides, sugar, cholesterol and the nitrogenous products of the blood?

This study includes about one hundred examinations of the blood made upon eighty-one individuals (Chart No. 1). We are unable to explain the difference between the average number of red cells in the normal bloods of our series and the normal bloods

reported by others. The fact remains, however, that a count of five million is a rare exception rather than the rule, with a large per cent running from four to four and a half million. Each of these counts, as well as hemoglobin determinations, has been made independently by two different workers in different laboratories, using standardized equipment, and discarding results when the two counts did not come within the average limits of error. Furthermore, the work was done by skilled hematologists and not by the usual laboratory technicians. The experience of other reliable laboratories convinces us that in Kentucky at least a normal red cell count of five million is the exception and the average is from 4.3 to 4.5 million, depending largely upon the source of the normals.

Greater accuracy attends the proper determination of the hemoglobin when the method used has been carefully checked with the accurate oxygen capacity method of Van Slyke,³ or the iron method of Kennedy or Wong. Inaccuracy of various methods has been stressed by Osgood and Haskins,² Karshan and Freeman,⁴ Haden,¹ Lindsay Rice and Sellinger,⁵ Robscheit,⁶ and others. The chief objection to the acid hematin methods seemed to lie in the time required for development of the color, fading of standards, variation in standard discs, and difficulty in reading small quantities of hemoglobin. The Osgood and Haskins is probably the most accurate of the acid hematin methods (see footnote), provided the standard solution is properly prepared and the proper corrections made for variations in temperature. The Palmer⁷ method is quite accurate if the standard is frequently checked. The photoelectrometer of Sanford and Sheard⁸ and the spectrophotometer of Haden⁹ are apparently accurate methods, checking closely with the oxygen capacity method or the iron method. Although quantitative determinations of iron in the blood were made as early as 1820¹⁰ and at long intervals following that, the most recent by Berman in 1918,¹¹ Brown in 1922,¹² Evehjen and Hart in 1926,¹³ Wong in 1923-1928,¹⁴ and Kennedy in 1927,¹⁵ the method received little attention until its clinical application by Powers and Murphy.¹⁶

Of the methods of Wong¹⁴ and Kennedy¹⁵ the latter points out that Wong, like others, has overlooked the question of dissociation and continued to work with aqueous solutions. The results, however,

by the two methods check so very closely that the criticism may well be ignored.

Using Kennedy's¹⁵ method Powers and Murphy¹⁶ found the amount of iron in the blood to be within singularly narrow and standard limits for normal adults. They consider it the most accurate criterion yet devised for evaluating the true status of patients with secondary anemia. In a later article Murphy, Lynch and Howard¹⁷ reported a large number of blood iron determinations in normal individuals, and also in the primary and secondary anemias, as well as a variety of other conditions. For normal young men they found an average of 44.84 and for young women an average of 42.84 mg. per 100 c.c. of blood. Using the same method we find very close agreement with an average of 44.0 for all normal cases. As would be expected, the results parallel closely the hemoglobin curve in all cases. They also suggested the term "iron index" (obtained by dividing the mg. of iron in 100 c.c. of blood by the number of red blood cells in millions per 1 c.mm. of blood) to represent the amount of iron in the individual red cell. They found for this an average of 8.4 (strictly speaking $8.4 \text{ mg.} \times 10^{-11}$) for normals, and following in a general way the color index curve. Since our red blood counts are lower on an average, the average normal index is higher, being as consistently 10.0 in our series as the 8.4 of their series (Charts Nos. 2, 4, 7 and 8).

Both Kennedy¹⁵ and Wong¹⁴ have used the iron content of the blood for accurately determining the amount of hemoglobin. When it is possible to obtain results for the iron content of tissue or fluids, that are accurate to the third decimal and with the molecular weight of the hemoglobin molecule known (though the structure may be in doubt), it becomes immediately apparent that the quantity of iron expressed in milligrams divided by 3.35 (Wong) or 3.34 (Kennedy) will accurately represent the quantity of hemoglobin in grams per 100 c.c. of blood. The work of Riecker,¹⁸ however, seems to prove that in normal individuals there is an average of 1.1 mgs. ($\pm .022$) of iron in the blood serum, and this quantity is increased in pernicious anemia particularly and slightly decreased in certain other conditions. In our study we have assumed (possibly wrongly) that cognizance of this non-hemoglobin iron has been ignored in the methods of Kennedy

and Wong and we have, therefore, subtracted 1 mg. from the total iron content of the blood and then multiplied the result by .3 (equal to dividing by .333 1-3, instead of the .334 and .335 used by Kennedy and Wong respectively). While this method makes a difference of less than two per cent, it has been used with the belief that accuracy was increased to this extent. The oxygen capacity method of Van Slyke³ compares so very closely with both the Wong¹⁴ and Kennedy¹⁵ methods that little or no doubt should be entertained as to the accuracy of any of the three methods. Contrary to the general belief, we consider both iron methods quite simple and easily completed in about fifteen minutes. We prefer, however, the Kennedy method¹⁵ because it allows time for other work while the iron mixture is being digested and, furthermore, the colorimetric readings are definitely sharper. Any of the three methods may be safely used for standardizing hemoglobinometers and checking the accuracy of other methods.

In our experience, as well as in the experience of others, the Osgood and Haskins² acid hematin method gives results closely paralleling the results of any of the three standard methods. We believe, therefore, that a determination of the iron content of the blood, the iron content of the individual cell and the determination of the hemoglobin by the iron method are all most valuable additions to our knowledge of hematology. They assume no averages and deal directly with the number of red blood cells and the hemoglobin found in the individual specimen. Of similar importance is the determination of the amount of hemoglobin in the individual cell. This is done by dividing the hemoglobin in grams per 100 c.c. of blood by the red cells in millions per c.mm. of blood. The result is expressed in grams times 10^{-12} , or in billionths of a milligram per red cell, according to the following formula:

$$\frac{\text{Hb. in gms.} \times 10}{\text{red cells in millions}}$$

Example: R.B.C. 3,500,000, Hb. 10.5 gms.

$$\frac{10.5 \times 10}{3.5} = \frac{105}{3.5} = 30 \text{ billionths of a mgm. per R.B.C.}$$

(or 30×10^{-9} mgm.)

The color index, determined in our series by the method of Cullen,¹⁹ cannot be quite so accurate since it assumes five million

red cells to be normal, and this factor enters into the equation. The results, however, conform closely with our general knowledge of what this should be, namely, high in pernicious anemia and low in secondary anemia.

It is our belief that a properly reported blood examination should contain the following data: (1) sufficient counts of the red blood cells and the white blood cells until they check within the accepted limits of error; (2) hemoglobin determinations by the iron method of Wong¹⁴ or Kennedy,¹⁵ or the acid hematin method of Osgood and Haskins;² (3) the average amount of hemoglobin per red cell; (4) the blood iron in milligrams per 100 c.c. of blood; (5) the iron index; (6) a supravital stain of the white cells as well as examination of the stained smear (this we believe should never be omitted and furnishes information not obtainable from the stained smear); (7) a platelet count; (8) reticulocyte count, and (9) interpretation of the findings and explanation of various hemoglobin standards (Chart No. 9). Thus it will be seen that a complete hematologic study is far from the simple procedure that has been used for years and impossible of accomplishment in the ten minutes that has been allotted and boasted for it. Most technical procedures in the laboratory have been greatly simplified without affecting their accuracy, but with out increasing knowledge of the blood, the procedure, if accurate, is becoming more difficult and more time-consuming, which is regrettable from many standpoints, the least of which is by no means financial. Because of the time consumed and the skill required it should not be included along with a urinalysis in a so-called routine examination, but accredited the same dignity that is accorded other special procedures.

CHART No. 1.
BLOOD STUDIES IN EIGHTY-ONE CASES WITH A VARIETY OF CONDITIONS.

Case	Sex	Age	Different Laboratories		Blood Iron	From Iron	Hemoglobin Determinations		Color Index		Diagnosis
			R.B.C.	R.B.C.			O. & H.	N.	Hb.	Iron Index	
1	F	36	4,096	4,340	43.2	12.7	12.0	13.0	.93	10.5	Normal.
2	M	41	4,312	4,670	46.5	13.6	13.6	13.9	.93	10.3	Normal.
3	F	45	4,200	41.5	12.1	12.187	9.8	Syphilis.
4	M	69	4,430	4,310	48.5	14.3	14.3	13.2	.98	10.9	Cholecystitis.
5	F	53	4,232	42.6	12.5	12.587	10.0	Hypertension.
6	F	24	4,240	4,510	39.8	11.6	11.5	11.8	.82	9.4	Normal.
7	M	64	4,536	47.5	13.9	14.091	10.4	Normal.
8	M	54	4,720	4,355	48.4	14.2	14.2	12.9	.90	10.2	Normal.
9	M	45	4,312	4,445	43.0	12.6	12.3	13.1	.87	10.0	Neurosis.
10	M	57	4,304	4,321	43.0	12.6	12.8	13.3	.87	10.0	Hypertension.
11	F	27	4,496	4,300	45.4	13.4	13.3	12.8	.90	10.1	Hydronephrosis.
12	F	34	4,480	4,470	41.6	12.1	12.0	13.3	.81	9.0	Neurosis.
13	F	67	4,680	4,700	47.0	13.8	14.0	13.6	.88	10.0	Normal.
14	F	65	4,408	4,360	42.8	12.5	12.8	13.3	.84	10.6	Hypertension.
15	F	35	4,256	4,320	42.5	12.4	12.4	12.2	.87	10.0	Surg. menopause.
16	M	58	4,208	4,120	43.5	12.8	13.1	12.4	.90	10.3	Hypertension.
17	F	20	4,576	4,520	46.8	13.7	14.0	13.2	.89	10.4	Normal.
18	F	22	4,288	4,350	41.3	12.1	12.3	13.0	.88	9.8	Normal.
19	F	53	4,096	4,363	42.9	12.6	12.8	12.9	.90	10.2	Normal.
20	F	55	4,208	4,376	40.8	11.9	12.1	13.0	.84	9.5	Arthritis.
21	F	47	4,480	4,350	48.0	14.1	14.0	13.3	.93	10.9	Migraine.
22	F	27	4,384	4,380	44.5	13.1	13.4	13.2	.88	10.3	Normal.
23	M	47	4,800	46.7	13.7	13.885	9.7	Renal calculus; bronchiectasis.
23	M	47	4,104	39.6	11.6	12.0	After nephrectomy.
24	F	43	3,904	35.4	10.3	10.378	9.0	Nutritional edema.
25	F	60	4,470	55.0	16.5	...	13.1	1.11	12.3	Adenocarcinoma ovary.
26	F	22	4,336	37.5	11.0	11.275	8.6	Normal.
27	F	19	4,280	40.0	11.7	11.982	9.3	Normal.

Case	Sex	Age	Differential Laboratories		Hemoglobin Determinations			Color Index		Diagnosis
			R.B.C.	R.B.C.	Blood Iron	From Iron	O. & H.	N.	Hb. RBC x 3	
28	F	39	4,396	35.7	10.4	10.772	8.1
29	F	57	2,896	2,970	30.0	8.7	8.3	8.3	.90	10.9
30	F	20	4,608	45.8	13.4	14.087	9.9
31	M	15	2,728	2,770	27.7	8.0	8.0	7.5	.87	10.0
32	F	55	4,112	4,270	43.0	12.6	12.2	12.2	.91	10.4
33	F	..	3,592	3,400	30.8	8.9	8.7	9.2	.75	8.6
33	F	..	3,880	4,260	34.5	10.0	9.9	13.0	.78	8.9
34	F	60	3,784	3,784	37.5	11.0	11.1	11.6	.87	9.9
35	M	8	2,240	22.2	6.4	6.185	9.9
36	M	12	4,048	39.9	11.7	11.986	9.8
37	F	51	3,280	3,490	32.0	9.3	9.0	9.0	.72	8.3
38	F	2 wks.	2,984	39.5	11.5	11.0	...	1.16	13.7
39	F	55	4,400	42.8	12.5	12.085	9.7
40	M	40	3,520	3,520	35.1	10.2	10.0	12.0	.87	10.0
41	M	52	5,696	4,940	55.5	16.3	15.9	14.9	.85	9.7
42	F	27	4,128	4,332	41.4	12.0	12.0	12.9	.87	10.0
43	F	28	4,280	4,200	40.0	11.7	12.2	12.7	.81	9.5
44	F	59	4,328	4,450	45.5	13.4	14.4	13.6	.91	10.6
45	F	71	4,032	4,100	42.5	12.4	12.6	12.8	.91	10.6
46	M	68	4,304	4,480	41.5	12.1	11.3	13.1	.84	9.6
47	F	65	4,800	49.0	14.4	14.590	10.2
48	F	43	4,160	41.6	12.2	12.287	10.0
49	F	..	4,080	46.2	13.6	13.899	11.3
50	F	24	3,840	3,810	37.4	10.9	10.4	12.4	.93	9.7
51	F	60	4,224	4,280	42.2	12.3	12.0	12.8	.87	10.0
52	F	29	4,280	4,320	40.4	11.8	12.0	12.9	.82	9.4
53	M	48	5,120	5,500	53.0	15.6	15.4	13.5	.90	10.3
54	F	51	4,608	4,330	46.5	13.6	13.6	13.5	.87	10.0
55	M	50	4,488	45.1	13.2	13.2	14.6	.88	10.5

Normal.
Acute myelogenous leukemia (autopsy).

Normal.
Hemorrhagic nephritis (autopsy).
Arthritis; focal infection.

Banti's disease.
After splenectomy.

Healed ulcer; pyloric obstruction.
Sickle cell anemia.

Typhoid.
Chr. lymphatic leukemia; lobar pneumonia.

Hydrocephalus secondary anemia.
Myocarditis with mitral insufficiency;
focal infection.

Syphilis, secondary.
Hypertension; gastro-enterostomy.
Thrombo-angitis.

Trichiniasis.
Pernicious anemia (remission).
Arsenical neuritis.

Cirrhosis of liver; myocarditis.
Endarteritis obliterans; gangrene.
Cholecystitis; mild hypothyroidism.

Colitis; focal infection.
Acoustic tumor.
Pelvic pathology.

Diverticulitis colitis.
Recurrent hyperthyroidism.
Duodenal ulcer.

56	F	35	3,704	43.4	42.7	11.0	...	1.02	11.7	Arthritis deformans.
57	M	58	4,728	5,510	48.8	14.3	14.4	15.3	.90	10.4	Duodenal ulcer.
58	F	37	3,856	4,300	40.0	11.7	11.7	12.8	.90	10.5	Colitis; neurosis.
59	M	20	5,056	4,480	51.0	15.0	15.3	14.1	.88	10.2	Chronic nephritis.
60	F	75	4,200	42.8	12.5	12.890	10.0	Arthritis.
61	M	43	5,536	4,510	51.0	15.0	15.1	13.6	.81	9.2	Tumor epididymis; hemorrhoids.
62	F	32	4,480	4,340	45.5	13.4	13.6	12.9	.90	10.1	Arthritis—spine.
63	F	58	4,392	4,370	43.0	12.6	13.0	13.0	.85	10.0	Auricular fibrillation.
64	M	77	4,336	4,321	41.0	12.0	12.0	13.0	.81	9.5	Auricular fibrillation; diabetes.
65	M	34	4,640	4,485	46.6	13.7	14.0	13.3	.87	10.0	Neurosis; duodenal obstruction.
66	F	61	4,480	4,350	45.4	13.3	13.4	12.8	.88	10.0	Uterine fibroid; cholecystitis.
67	M	75	992	1,020	14.3	4.0	4.0	5.5	1.20	14.4	Pernicious anemia.
68	F	53	3,896	3,910	38.8	11.3	11.6	12.0	.87	10.0	Secondary anemia.
69	F	53	4,592	4,430	45.0	13.2	13.3	13.2	.87	10.0	Arterial hypertension.
70	M	60	1,792	1,760	21.9	6.3	6.4	4.8	1.05	12.2	Pernicious anemia.
71	M	65	3,520	3,000	39.0	11.4	11.6	7.07	.97	11.0	Pernicious anemia.
72	M	55	1,994	1,960	29.5	8.6	8.8	5.9	1.29	14.3	Pernicious anemia.
73	F	18	2,688	2,850	23.3	6.7	6.9	5.5	.74	8.7	Chlorosis.
74	M	70	4,640	4,410	46.1	13.5	13.9	14.0	.87	10.0	Nephritis—pneumonia—death.
75	F	29	4,384	4,500	43.8	12.8	13.2	13.2	.90	10.0	Cerebral lesion.
76	F	..	1,104	900	18.4	5.2	5.5	5.1	1.41	16.6	Pernicious anemia.
77	F	56	7,504	7,420	75.5	22.3	21.8	21.0	.88	10.0	
			7,840	7,620	72.5	21.4	21.0	21.0	.81	9.3	
			7,968	7,820	80.6	23.9	22.8	22.0	.90	10.2	Polycythemia vera.
			7,264	70.0	20.7	20.285	9.7	
			7,200	65.0	19.2	19.680	9.0	
			6,984	60.6	17.9	18.278	8.8	
			3,872	3,872	37.8	11.0	11.3	13.5	.87	9.7	
78	M	58	4,684	4,940	46.5	13.6	13.9	14.5	.87	10.0	Normal.
79	F	48	4,560	4,439	45.0	13.2	13.6	13.3	.87	10.0	Normal.
80	M	..	3,520	3,525	37.0	10.8	11.0	11.5	.91	10.5	Bleeding duodenal ulcer.
81	M	32	3,600	4,375	35.7	10.4	10.6	13	.87	9.9	Acute lead poisoning.

CHART No. 2.

NORMAL BLOODS.											
Case	Sex	Age	Different Laboratories		Blood		Hemoglobin Determinations		Color Index Hb.		Diagnosis
			R.B.C.	R.B.C.	Iron	From Iron	O. & H.*	N.*	RBCx3 Index	Iron Index	
1	F	36	4,096	4,340	43.2	12.7	12.0	13.0	.93	10.5	Normal.
2	M	41	4,312	4,670	46.5	13.6	13.6	13.9	.93	10.3	Normal.
3	F	45	4,200	41.5	12.1	12.187	9.8	Syphilis.
4	M	69	4,430	4,310	48.5	14.3	14.3	13.2	.98	10.9	Cholecystitis.
5	F	53	4,232	42.6	12.5	12.587	10.0	Hypertension.
6	F	24	4,240	4,510	39.8	11.6	11.5	11.8	.82	9.4	Normal.
7	M	64	4,536	47.5	13.9	14.091	10.4	Normal.
8	M	54	4,720	4,355	48.4	14.2	14.2	12.9	.90	10.2	Normal.
9	M	45	4,312	4,445	43.0	12.6	12.3	13.1	.87	10.0	Neurosis.
10	M	57	4,304	4,321	43.0	12.6	12.8	13.3	.87	10.0	Hypertension.
11	F	27	4,496	4,300	45.4	13.4	13.3	12.8	.90	10.1	Hydronephrosis.
12	F	34	4,480	4,470	41.6	12.1	12.0	13.3	.81	9.0	Neurosis.
13	F	67	4,680	4,700	47.0	13.8	14.0	13.6	.88	10.0	Normal.
14	F	55	4,408	4,360	42.8	12.5	12.8	13.3	.84	10.6	Hypertension.
15	F	35	4,256	4,320	42.5	12.4	12.4	12.2	.87	10.0	Surg. menopause.
16	M	58	4,208	4,120	43.5	12.8	13.1	12.4	.90	10.3	Hypertension.
17	F	20	4,576	4,520	46.8	13.7	14.0	13.2	.89	10.4	Normal.
18	F	22	4,288	4,350	41.3	12.1	12.3	13.0	.88	9.8	Normal.
19	F	53	4,096	4,363	42.9	12.6	12.8	12.9	.90	10.2	Normal.
20	F	55	4,208	4,376	40.8	11.9	12.1	13.0	.84	9.5	Arthritis.
21	F	47	4,480	4,350	48.0	14.1	14.0	13.3	.93	10.9	Migraine.
22	F	27	4,384	4,380	44.5	13.1	13.4	13.2	.88	10.3	Normal.
Average			4,361	4,397	44.1	12.93	12.97	13.0	.885	10.1	

*N.=Newcomer. O & H.=Osgood & Haskins.

Red blood cells expressed in millions per 1 c.c.; Blood iron in milligrams per 100 c.c.; Hemoglobin in gms. per 100 c.c.; Iron Index= $\frac{\text{Fe (gms.)}}{\text{R.B.C. (millions)}}$; Color Index= $\frac{\text{Hb. (gms.)}}{\text{R.B.C. (millions} \times 3)}$; Hemoglobin from iron calculated viz.: Mgs. blood iron per 100 c.c.—1 mg. serum iron $\times .3$.

CHART No. 3.

COMPARISON OF VARIOUS HEMOGLOBIN METHODS AND THE CLOSE RELATIONSHIP
BETWEEN METHODS OF WONG AND KENNEDY FOR BLOOD
IRON DETERMINATIONS.

Blood Iron Mg. per 100 c.c.			Hemoglobin Gms. per 100 c.c. Blood			
Wong	Kennedy	Difference	Wong	Kennedy	Osgood & Haskins	—1 x .3
			Blood Iron .335	Blood Iron .334		
45.6	45.4	—0.2	13.6	13.5	13.4	13.3
13.8	14.3	+0.5	4.1	4.3	4.0	4.0
38.7	38.8	+0.1	11.5	11.6	11.6	11.3
39.4	39.0	—0.4	11.7	11.6	11.6	11.4
29.7	29.5	—0.2	8.8	8.6	8.8	8.5
18.4	18.4	0	5.5	5.5	5.5	5.2
37.1	37.0	—0.1	11.1	11.1	11.0	10.8
Average		—0.043		—0.014	—0.057	

HEMOGLOBIN (GRAMS PER 100 C.C.) LINDSAY, RICE & SELLINGER.

Van Slyke	Wong	Difference	Newcomer	Sahli	Dare A
18.78	18.80	+0.02	18.5	18.7	...
15.89	15.79	—0.10	15.2	18.9	10.3
15.16	15.87	+0.71
14.15	14.85	+0.70
12.21	12.48	—0.27	9.1	15.0	8.7
9.06	8.13	—0.93	9.1	15.7	6.8
7.88	7.89	+0.01	9.3	15.7	7.0
Average		+0.097			

HEMOGLOBIN (GRAMS PER 100 C.C.) KARSHAN & FREEMAN.

Van Slyke (Means)	Wong (Means)	Cohen & Smith (Means)	Difference
12.55	12.65	+0.10
12.55	12.35 (finger)	—0.20
12.55	12.45 (vein)	—0.10
....	12.65	12.35 (finger)	—0.30
....	12.65	12.45 (vein)	—0.20

CHART No. 4.
PRIMARY DISEASES OF THE BLOOD.

Case	Sex	Age	Differential Laboratories		Blood Iron	Color Index	Hemoglobin Index	Hemoglobin Determinations		From Iron	Diagnosis
			R.B.C.	R.B.C.				N.*	O. & H.*		
29	M	57	2,896	2,970	30.0	10.9	90	8.3	8.3	8.7	Acute myelogenous leukemia (autopsy).
33	F	61	3,592	3,400	30.8	8.6	.75	9.2	8.7	8.9	Banti's disease.
33	F	61	3,880	4,260	34.5	8.9	.78	13.0	9.9	10.0	After splenectomy.
35	M	8	2,240	22.2	9.9	.85	...	6.1	6.4	Sickle cell anemia.
44	F	59	4,328	4,450	45.4	10.6	.91	13.6	14.4	13.4	Pernicious anemia (remission).
45	F	71	4,032	4,100	42.3	10.6	.91	12.8	12.6	12.4	Pernicious anemia (remission).
67	M	75	992	1,020	14.3	14.4	1.20	5.5	4.0	4.0	Pernicious anemia.
70	M	60	1,792	1,760	21.9	12.2	1.05	4.8	6.4	6.3	Pernicious anemia.
71	M	65	3,520	3,000	39.0	11.0	.97	7.07	11.6	11.4	Pernicious anemia.
72	M	55	1,994	1,960	29.5	14.3	1.29	5.90	8.8	8.6	Pernicious anemia.
76	F	..	1,104	18.4	16.6	1.41	...	5.5	5.2	Pernicious anemia.
73	F	18	2,688	2,850	23.3	8.7	.74	5.5	6.9	6.7	Chlorosis.
37	F	51	3,280	3,490	32.0	8.3	.72	9.0	9.0	9.3	Chronic lymphatic leukemia — lobar pneumonia.
77	F	56	7,504	7,420	75.5	10.0	.88	21.0	21.8	22.3	Polycythemia vera.
			7,840	7,620	72.5	9.3	.81	21.0	21.0	21.4	Phenylhydrazine grs. iv daily.
			7,968	7,820	80.6	10.2	.90	22.0	22.8	23.9	Determinations weekly intervals.
			7,264	70.0	9.7	.85	...	20.2	20.7	
			7,200	65.0	9.0	.80	...	19.6	19.2	
			6,984	60.6	8.8	.78	...	18.2	17.9	
			3,872	3,872	37.8	9.7	.87	13.5	11.3	11.0	

*N.=Newcomer. O. & H.=Osgood & Haskins.

Red blood cells expressed in millions per 1 c.c.; Blood iron in milligrams per 100 c.c.; Hemoglobin in gms. per 100 c.c.; Iron Index=Fe. (gms.) ÷ R.B.C. (millions); Color Index=Hb. (gms.) ÷ R.B.C. (millions) x 3; Hemoglobin from iron calculated, viz.: Mgs. blood iron per 100 c.c.—1 mg. serum iron x .3.

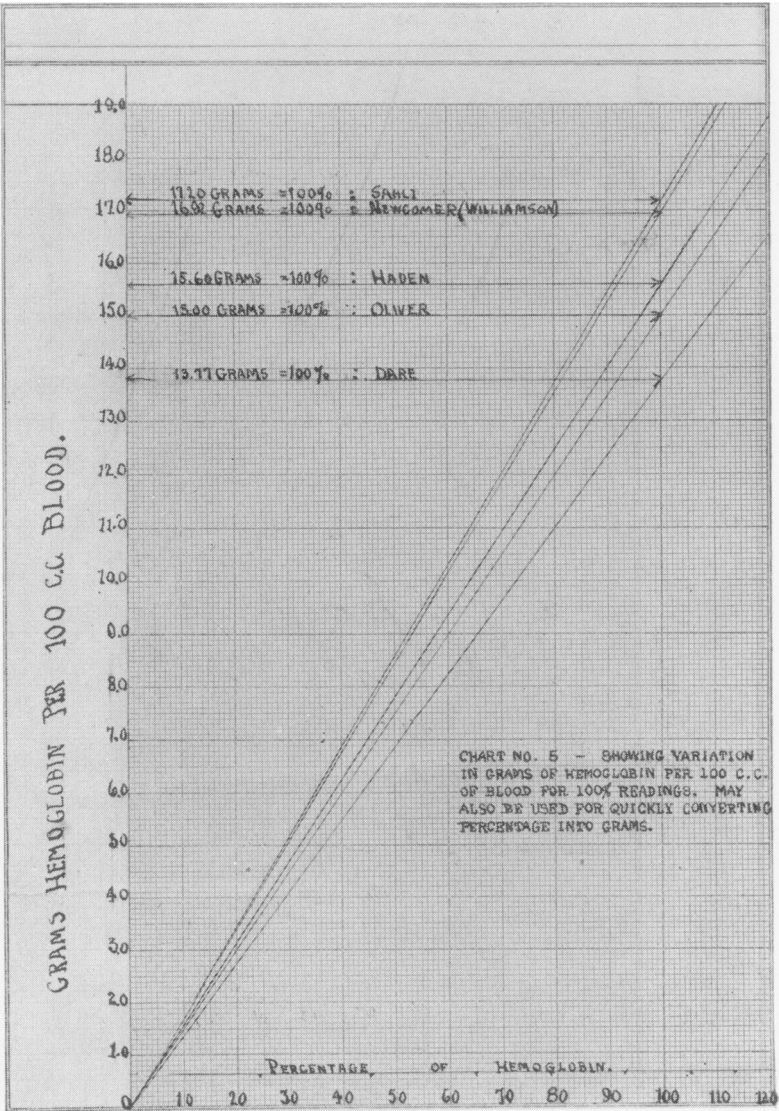


CHART No. 5.

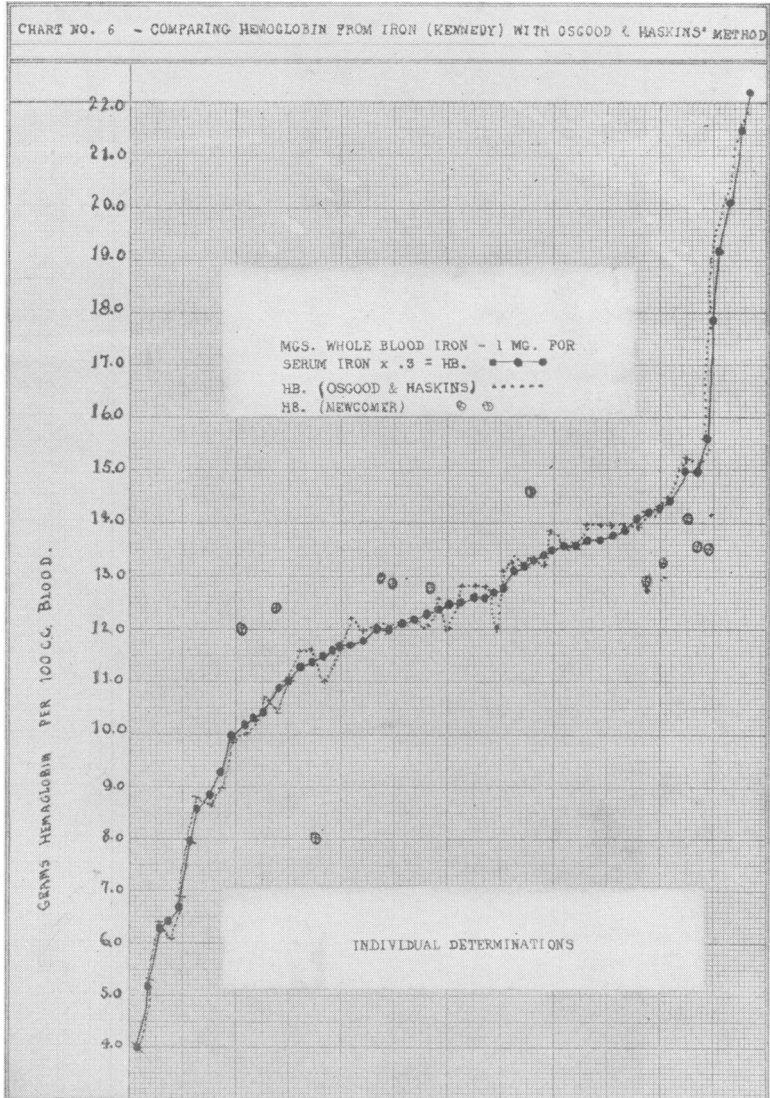


CHART No. 6.

CHART No. 7.

DISEASES WITH ABNORMAL BLOOD.

Case	Sex	Age	Different Laboratories		Blood Iron	Color Index		Hemoglobin Determinations		From Iron	Diagnosis
			R.B.C.	R.B.C.		RBCx3	Hb.	N*	O. & H.*		
24	F	43	3,904	35.4	9.0	.78	...	10.3	10.3	Nutritional edema.
31	F	15	2,728	2,770	27.7	10.0	.87	7.5	8.0	8.0	Hemorrhagic nephritis (autopsy).
34	F	60	3,784	3,784	37.5	9.9	.87	11.6	11.1	11.0	Duodenal ulcer; pyloric obstruction.
38	F	2 wks.	2,984	39.5	13.7	1.16	...	11.0	11.5	Hydrocephalus secondary anemia.
40	M	40	3,520	3,520	35.1	10.0	.87	12.0	10.0	10.2	Cerebrospinal syphilis, secondary stage.
74	M	70	4,640	4,410	46.1	10.0	.87	14.0	13.9	13.5	Nephritis; pneumonia.
50	F	24	3,840	3,810	37.4	9.7	.93	12.4	10.4	10.9	Colitis; focal infection.
58	F	37	3,856	4,300	40.0	10.5	.90	12.8	11.7	11.7	Colitis; neurosis.
68	F	53	3,896	3,910	38.8	10.0	.87	12.0	11.6	11.3	Secondary anemia.
80	M	47	3,520	3,525	37.0	10.5	.91	11.5	11.0	10.8	Duodenal ulcer; bleeding.

*N.=Newcomer. O. & H.=Osgood & Haskins.

Red blood cells expressed in millions per 1 c.c.; Blood iron in milligrams per 100 c.c.; Hemoglobin in gms. per 100 c.c.; Iron Index=Fe. (gms.)÷R.B.C. (millions); Color Index=Hb. (gms.)÷R.B.C. (millions) x 3; Hemoglobin from iron calculated, viz.: Mgs. blood iron per 100 c.c.—1 mg. serum iron x .3.

CHART No. 8.

DISEASES WITH NORMAL BLOOD.

Case	Sex	Age	Differential Laboratories		Blood Iron	Color Index	Hemoglobin Determinations			Diagnosis	
			R.B.C.	R.B.C.			N.*	O. & H.*	From Iron		
23	M	47	4,800	46.7	9.7	.85	...	13.8	13.7	Renal calculus; bronchiectasis.
25	F	60	4,470	55.0	12.3	1.11	13.1	...	16.5	Adenocarcinoma ovary.
32	F	55	4,112	4,270	43.0	10.4	.91	12.2	12.2	12.6	Arthritis; focal infection.
36	M	12	4,048	39.9	9.8	.86	...	11.9	11.7	Typhoid fever.
39	F	55	4,400	42.8	9.7	.85	...	12.0	12.5	Myocarditis; mitral insufficiency; focal infection.
41	M	52	5,696	4,940	55.5	9.7	.85	14.9	15.9	16.3	Hypertension; gastro-enterostomy.
42	F	27	4,128	4,332	41.4	10.0	.87	12.9	12.0	12.0	Thrombo-angiitis obliterans.
43	F	28	4,280	4,200	40.0	9.5	.81	12.7	12.2	11.7	Trichiniasis.
46	M	68	4,304	4,480	41.5	9.6	.84	13.1	11.3	12.1	Cirrhosis of liver; myocarditis.
47	F	65	4,800	49.0	10.2	.90	...	14.5	14.4	Enderteritis obliterans; gangrene.
48	F	43	4,160	41.6	10.0	.87	...	12.2	12.2	Cholecystitis; mild hyperthyroidism.
51	F	60	4,224	4,280	42.2	10.0	.87	12.8	12.0	12.3	Acoustic tumor.
52	F	29	4,280	4,320	40.4	9.4	.82	12.9	12.0	11.8	Pelvic pathology.
53	M	48	5,120	5,500	53.0	10.3	.90	13.5	15.4	15.6	Diverticulitis colitis.
54	F	51	4,608	4,330	46.5	10.0	.87	13.5	13.6	13.6	Recurrent hyperthyroidism.
55	M	50	4,488	45.1	10.5	.88	14.6	13.2	13.2	Duodenal ulcer.
57	M	58	4,728	5,510	48.8	10.4	.90	15.3	14.4	14.3	Chronic nephritis.
59	M	20	5,056	4,480	51.0	10.2	.88	14.1	15.3	15.0	Arthritis.
60	F	75	4,200	42.8	10.0	.90	...	12.8	12.5	Arthritis.
62	F	32	4,480	4,340	45.5	10.1	.90	12.9	13.6	13.4	Auricular fibrillation.
63	F	58	4,392	4,370	43.0	10.0	.85	13.0	13.0	12.6	Auricular fibrillation—diabetes.
64	M	77	4,336	4,321	41.0	9.5	.81	13.0	12.0	12.0	Duodenal stasis; neurosis.
65	M	34	4,640	4,485	46.6	10.0	.87	13.3	14.0	13.7	Uterine fibroid; cholecystitis.
66	F	61	4,480	4,350	45.4	10.0	.88	12.8	13.4	13.3	Arterial hypertension.
69	F	53	4,592	4,430	45.0	10.0	.87	13.2	13.3	13.2	Nephritis—pneumonia (death in 36 hrs.).
74	M	70	4,640	4,410	46.1	10.0	.87	14.0	13.9	13.5	Cerebral lesion.
75	F	29	4,384	4,500	43.8	10.0	.90	13.2	13.2	12.6	
Average			4,514	4,515	45.2	10.0	.877	13.38	13.19	13.28	

*N.=Newcomer. O. & H.=Osgood & Haskins.

Red blood cells expressed in millions per 1 c.c.; Blood iron in milligrams per 100 c.c.; Hemoglobin in gms. per 100 c.c.; Iron Index= $\frac{\text{Fe. (gms.)} \div \text{R.B.C. (millions)}}{\text{Iron calculated, viz.: Mgs. blood iron per 100 c.c.} \div 1 \text{ mg. serum iron} \times 3}$; Color Index= $\frac{\text{Hb. (gms.)} \div \text{R.B.C. (millions)}}{\text{Hemoglobin from iron calculated, viz.: Mgs. blood iron per 100 c.c.} \div 1 \text{ mg. serum iron} \times 3}$.

CHART No. 9.

A ROUTINE REPORT OF A BLOOD EXAMINATION (MARTIN-McNEILL
LABORATORIES).

13925. Mr. H.B.

April 5, 1932.

BLOOD EXAMINATION.

R. B. C.	2,896,000—2,970,000
Hemoglobin (Osgood & Haskins)	9.0 gm. per 100 c.c. blood (normal about 15)*
Average amount per red cell .	31 billionths of a mg. (normal about 30)*
Blood iron	30 mg. per 100 c.c. blood (normal about 46)
Iron Index	10.3
W. B. C.	20,050—21,050

DIFFERENTIAL.

	Romanowsky Stain %	Supravital Stain %	No. per c.mm.
Myeloblasts	45.0	69.5	13900
Promyelocytes	1.0	2.0	400
Neutrophilic myelocytes . . .	1.0	4.5	900
Eosinophilic myelocytes . . .	0.0	0.0	0
Basophilic myelocytes	0.0	0.5	100
Metamyelocytes	1.0	1.5	300
P. M. neutrophiles	9.5	7.5	1500
P. M. eosinophiles	0.0	0.0	0
P. M. basophiles	0.0	0.5	100
Monocytes	1.5	6.0	1200
Lymphocytes	2.0	7.0	1400
Immature lymphocytes	0.0	1.0	200
Smudges	39.0	0.0	0
	100.0%	100.0%	20,000
Platelet count		25,000	
Arneth's index		89 (normal about 60)	
Reticulocytes		10%	
Malarial parasites		None	

The number of red cells is considerably reduced, but the average amount of hemoglobin per red cell is normal, due to an increase in the average size of the cells. Many of the large cells are polychromatic, and a number of normoblasts and megaloblasts are present. There is very little distortion.

Thirty-nine per cent of the white cells are so fragile as to be destroyed in pulling a smear. These fragile cells are identified in the supravital preparation as myeloblasts. These cells have a large round nucleus with several nucleoli and a basophilic cytoplasm. All gradations between these non-granular cells and the typical adult neutrophile can be found.

Impression: Myeloblastic leukemia.

*The percentage of hemoglobin of this patient varies according to the normal standard of hemoglobin selected—for instance, with the Sahli standard it is 52% (color index 0.9), and with the Dare standard it is 66% (color index 1.2).

COLORIMETRIC DETERMINATION OF IRON AND HEMOGLOBIN
IN BLOOD.

By San Yin Wong, J. of Biological Chemistry, 1928, Vol. LXXVII.

Reagents Required:

1. Concentrated sulfuric acid.
2. Sodium tungstate. Dissolve an appropriate amount of good grade sodium tungstate to make a ten per cent solution.
3. Saturated potassium persulphate. Introduce into a small glass-stoppered bottle about 7 gms. of pure potassium persulphate and shake up with 100 c.c. of distilled water. The undissolved portion settles on the bottom to make good any decomposition upon standing.
4. Potassium sulfocyanate. Prepare approximately a 3N solution by dissolving 146 gms. of pure potassium sulfocyanate in distilled water to make 500 c.c. Filter if necessary. Add 20 c.c. of pure acetone to improve its keeping quality.
5. Standard iron solution. Weigh out accurately 0.7 gm. of crystallized ferrous ammonium sulfate and dissolve in about 50 c.c. of distilled water. Add to the solution 20 c.c. of dilute (10 per cent) iron free sulfuric acid, warm slightly, and then add 0.1 N (approximate) potassium permanganate solution to oxidize the ferrous salt completely. Dilute with distilled water to one liter. Each c.c. will contain 0.1 mg. of iron for use as a regular standard.

Procedure:

Transfer accurately with an Ostwald pipette 0.5 c.c. of blood into a 50 c.c. volumetric flask and introduce 2 c.c. of iron-free concentrated sulfuric acid. Whirl the flask to agitate the mixture for one or two minutes. Add 2 c.c. of saturated potassium persulfate solution and shake. Dilute to about 25 c.c. with distilled water and add 2 c.c. of 10 per cent sodium tungstate solution. Mix. Cool to room temperature under the tap and then dilute to volume with distilled water. Stopper the flask and invert two or three times to effect thorough mixing. Filter through a dry filter paper into a clear, dry receiving vessel. Pipette exactly 20 c.c. of the clear filtrate into a large test tube graduated at 20 and 25 c.c. Measure into another similar test tube 1 c.c. of the standard iron solution containing 0.1

mg. of Fe per c.c. Add with a graduated 1 c.c. pipette 0.8 c.c. of iron-free concentrated sulfuric acid and dilute to the 20 c.c. mark with distilled water. Cool to room temperature under the tap. Now add to both the unknown and standard 1 c.c. of saturated potassium persulfate and 4 c.c. of 3 N potassium sulfocyanate solution. Insert a clean rubber stopper, mix and compare in a Duboscq colorimeter.

Calculation:

As the 20 c.c. of filtrate taken represent 0.2 c.c. of blood and the quantity of standard solution used contains 0.1 mg. of Fe, if the reading is made with the standard set at 20 mm., then 20 divided by the reading (R) of the unknown and multiplied by 50 will give the number of mg. of Fe in 100 c.c. of blood. To obtain the percentage of hemoglobin, divide this number by 3.35, since hemoglobin contains 0.0335 per cent iron.

$$\frac{20 \times 50}{R} = \text{Mg. Fe per 100 c.c. blood.}$$

$$\frac{20 \times 50}{R \times 3.35} = \text{Hemoglobin in gms. per 100 c.c.}$$

THE QUANTITATIVE DETERMINATION OF IRON IN BLOOD.

R. P. Kennedy, J. Biol. Chem., 74, 385.

Standard Iron Solution: 0.5 gm. of pure iron wire is weighed and dissolved in 25 c.c. of 10 per cent sulfuric acid. After complete solution 3 c.c. of concentrated nitric acid is added to convert all of the iron to the ferric state. The solution is then transferred to a volumetric flask and made up to one liter. This is the standard stock solution, 1 c.c. of which contains 0.5 mg. of iron. The standard solution is prepared from the stock solution as follows: 10 c.c. of the stock solution is transferred to a liter volumetric flask and diluted to approximately 500 c.c. 20 c.c. of 60% perchloric acid and 50 c.c. of concentrated sulfuric acid are added and the solution made up to 1000 c.c. 1 c.c. of this standard contains .005 mg. of iron. The perchloric and sulfuric acids are added to the standard to counterbalance the acidity of the digested blood. It is very important, in the determination of iron by this method, that the acidity of the standard and unknown be of equal strength.

Digestion: 1 c.c. of blood is measured into a 100 c.c. Kjeldahl flask. 5 c.c. of concentrated sulfuric and 2 c.c. of 60% perchloric acids are added. The flask is then connected to a fume remover and digested until the solution is clear and colorless. This will require from ten to fifteen minutes. After cooling, a drop of nitric acid is added and the mixture diluted to 100 c.c.

Colorimetry: 10 c.c. aliquot portions of the solutions (standard and unknown) are measured into 50 c.c. glass-stoppered cylinders. 10 c.c. of amyl alcohol and 5 c.c. of 20% sodium sulfocyanate solution are added and the mixture shaken at once. The alcohol layer separates sharply and almost immediately and contains quantitatively the colored ferric sulfocyanate. The colored layer is removed with a pipette and transferred to a colorimeter cup and compared with the standard in a Duboscq colorimeter.

$$\frac{S}{X} \times 50 = \text{Mg. iron per 100 c.c. blood.}$$

S—reading of standard. X—reading of unknown.

$$\frac{\text{Gms. Fe per 100 c.c.}}{0.00334} = \text{hemoglobin in gms. per 100 c.c.}$$

ESTIMATION OF HEMOGLOBIN.

Osgood and Haskins, J. Biol. Chem., 1923, Vol. LVII.

Standard Solution: The standard used is an aqueous solution of ferric sulfate and was prepared by Hynson, Westcott and Dunning, Baltimore.

Technique: 0.05 c.c. of blood is measured with an accurate 0.1 c.c. pipette and transferred to a tube containing 2.45 c.c. of water. In cases of marked anemia use double quantities of blood. When the blood is completely hemolyzed add exactly 2.5 c.c. of 0.2 N HCl (18 c.c. of C.P. acid per liter). The volume of the mixture is 5 c.c. and the dilution of blood is one part in 100. Warm the tube in a water bath at 55-60° for seven minutes or more. This develops the maximum color of the acid hematin. Compare in the colorimeter with the standard ferric sulfate solution.

The standard is set at 15. The hemoglobin can then easily be determined by comparing the reading of the unknown with the chart given in the original article.

BIBLIOGRAPHY.

1. HADEN, R. I.: The Normal Hemoglobin Standard, J.A.M.A., 79:1496 (Oct. 28) 1922. Accurate Criteria for Differentiating Anemias, Arch. Int. Med., 31:766 (May) 1923.
The Technic of Determination of the Relative Mass, the Individual Cell Volume and the Volume Index of the Erythrocytes of Man, Jour. Lab. Clin. Med., 15:736 (May) 1930.
2. OSGOOD, E. E., AND HASKINS, H. D.: A New Permanent Standard for the Estimation of Hemoglobin by the Acid Hematin Method, Jour. Biol. Chem., 57:107, 1923.
OSGOOD, E. E.: Hemoglobin, Color Index, Saturation Index and Volume Index Standards, Arch. Int. Med., 37:685.
HOSKINS, E. E., AND HASKINS, H. D.: The Relation Between Cell Count, Cell Volume, and Hemoglobin Content of Venous Blood of Normal Young Women, Arch. Int. Med., 39:643.
3. VAN SLYKE AND NEILL: J. Biol. Chem., 61:523, 1924.
NEWCOMER, H. S.: J. Biol. Chem., 37:465, 1919.
BROWN, G. E., AND ROWNTREE, L. G.: The Volume and Composition of the Blood and the Changes Incident to Diuresis in Cases of Edema, Arch. Int. Med., 35:129 (Jan.) 1925.
KERN, R. A.: Problems of Clinical Hemoglobin Estimation, Med. Clin. N. Am., 8:821 (Nov.) 1924.
RICHARD, R. J., PIERCE, L. F., AND WORTHINGTON, R. L.: On Hemoglobinometry, J. Lab. Clin. Med., 15:746 (May) 1930.
COHEN, B., AND SMITH, A. H.: J. Biol. Chem., 39:489, 1919.
4. KARSHAN, M., AND FREEMAN, R. E., JR.: A Study of Hemoglobin Methods, J. Lab. Clin. Med., 15:74 (Oct.) 1929.
5. LINDSAY, RICE AND SELLINGER: A Plea for a Standardized Method of Estimating and Reporting Hemoglobin Values, J. Lab. Clin. Med., 11:737 (1925-26).
6. ROBSCHKEIT, FRIEDA S.: A Comparative Study of Hemoglobin Determinations by Various Methods, J. Biol. Chem., 41:2, P209.
7. PALMER, W. W.: J. Biol. Chem., 33:119, 1918.
8. SHEARD, C., AND SANFORD, A. H.: Photo-electrometer with One Stage of Amplification as Applied to Determination of Hemoglobin, J.A.M.A., 93:1951, Dec. 21, 1929.
SANFORD, A. H., AND SHEARD, C. S.: The Determination of Hemoglobin with the Photo-electrometer, J. Lab. Clin. Med., 15:483 (Feb.) 1930.
9. HADEN, R. L.: A New Hemoglobinometer, J. Lab. Clin. Med., 16:68 (Oct.) 1930.
10. LE CANU, PREVOST, AND DUMAS; quoted by MURPHY, LYNCH AND HOWARD: Arch. Int. Med., 47:885 (June) 1931.
11. BERMAN, L.: A Rapid Method for the Determination of Iron in Small Quantities of Blood, J. Biol. Chem., 35:231, 1918.
12. BROWN, A. L.: A New Quantitative Method for the Determination of Iron in the Blood, J. Am. Chem. Soc., 44:432, 1922.
13. ELVEHJEM, C. A., AND HART, E. B.: Serum Iron Determinations Applied to the Study of Experimental Anemias, Am. J. Physiol., 92:196 (Feb.) 1930.
14. WONG, S. Y.: J. Biol. Chem., 55:421, 1923. J. Biol. Chem., 77:409, 1928.
15. KENNEDY, R. P.: The Quantitative Determination of Iron in the Tissues, J. Biol. Chem., 74:385, 1927.
16. POWERS, J. H. AND MURPHY, W. P.: Treatment of Secondary Anemia, J.A.M.A., 96:504 (Feb. 14) 1931.
17. MURPHY, W. P., LYNCH, RALPH, AND HOWARD, ISABEL N.: The Value of Determinations of the Iron Content of Whole Blood, Arch. Int. Med., 47:883 (June) 1931.
18. RIECKER, HERMAN A.: Iron Metabolism in Pernicious Anemia and in Secondary Anemia, Arch. Int. Med., 46:458 (Sept.) 1930.
19. CULLEN, G. E.: Graphic Representation of Hemoglobin and Red Blood Cell Count Ratios, J.A.M.A., 98:1067, March 26, 1932.